Root growth, water relations, abscisic acid and proline levels of drought-resistant and drought-sensitive maize cultivars in response to water stress

B.P. O'Regan,*† W.A. Cress* and J. van Staden*

* UN/FRD Research Unit for Plant Growth and Development, Botany Department, University of Natal, P.O. Box 375, Pietermaritzburg 3200, Republic of South Africa

† Plant Biotechnology Research Centre, Private Bag X293, Pretoria 0001, Republic of South Africa

Received 24 December 1991; revised 14 May 1992

Two maize (*Zea mays* L.) cultivars, the one drought resistant (PNR473) and the other drought sensitive (SR52), were grown in a greenhouse under two watering treatments: a control treatment, in which plants were watered throughout, and a water stress treatment, in which plants were subjected to a period when watering was withheld. The response of growth and some physiological characteristics of the two cultivars were compared. The drought-resistant cultivar had a lower growth rate than the drought-sensitive cultivar in the control treatment, but had a higher growth rate and deeper rooting than the drought-sensitive cultivar in the water stress treatment. There were no differences between the cultivars in some physiological characteristics in the control treatment, but in the water stress treatment the drought-resistant cultivar had a higher transpiration rate and lower diffusive resistance during the onset of water stress, and higher relative water content and levels of abscisic acid and proline throughout the period of water stress.

Twee mielie- (*Zea mays* L.) kultivars, die een droogtebestand (PNR473) en die ander een droogte-sensitief (SR52), is by twee besproeiingsbehandelings gekweek. Die kontrolebehandeling is deurgaans met water voorsien maar in die droogtebehandeling is water vir 'n periode weerhou. Die effek op ontwikkeling en sommige fisiologiese eienskappe van die kultivars is vergelyk. Die droogtebestande kultivar het 'n stadiger groeitempo as die droogte-sensitiewe kultivar in die kontrolebehandeling gehandhaaf, maar het in die droogtestremmingsbehandeling 'n vinniger groeitempo gehandhaaf en 'n dieper wortelstelsel ontwikkel. Daar was geen fisiologiese verskille tussen die twee kultivars in die kontrolebehandeling nie, maar in die droogtestremmingsbehandeling het die droogtebestande kultivar 'n hoër transpirasietempo en 'n laer diffusieweerstand gehad tydens die beginfase van droogtestremming. Die kultivar het ook 'n hoër relatiewe waterinhoud, absisiensuur- en prolien-inhoud gehandhaaf gedurende die hele waterstremmingsperiode.

Keywords: *Zea mays* L.; water stress; roots; growth; water relations; abscisic acid; proline.

Introduction

Genotypic differences occur in the growth response of maize (*Zea mays* L.) to water stress (Hall *et al.* 1981; Lorens *et al.* 1987; Sobrado 1990). Although genotypic differences in response to water stress have also been identified for a range of morphological and physiological characteristics, including root development (Hurd 1974), stomatal activity (Beadle *et al.* 1973; Beardsell & Cohen 1975; Ackerson *et al.* 1979; Quarrie 1980; Ackerson 1983), osmotic adjustment (Ackerson *et al.* 1979), abscisic acid (Beardsell & Cohen 1975; Quarrie 1980; Ackerson 1983) and proline levels (Blum & Ebercon 1976; Hanson *et al.* 1977; Quarrie 1980; Thakur & Rai 1981), it is uncertain which characteristics are important in maintaining growth under conditions of water stress.

This study was undertaken to compare the response to water stress of two maize cultivars which differ in their resistance to drought. Comparisons were made with respect to root and leaf growth, stomatal activity, water relations, abscisic acid and proline levels. The plants were water-stressed by soil-drying in preference to other methods, since this would more accurately reflect what happens in the field.

Materials and Methods

Study plants

The two *Zea mays* L. cultivars chosen for study were PNR473, a drought-resistant cultivar, and SR52, a drought-sensitive cultivar.

Leaf and root growth

Plants were grown in a 1:1 mix of sieved compost and sand to which approximately 20 g l^{-1} of 2:3:2/N:P:K had been added. A single mix of soil was used for the experiment. The soil was contained in vertical tubes of PVC, each 10 cm in diameter and 60 cm in depth, and cut longitudinally with the two halves reattached with masking tape. Each tube was perforated at the base and at 10-cm intervals on opposite sides to allow drainage and aeration of the soil. After filling, the soil was watered to saturation and allowed to settle, whereupon more soil was added and rewatered. This was repeated until the soil remained level with the top of the tube. Vermiculite was sprinkled on the soil surface to keep it moist.

Seeds of both cultivars were germinated in seed trays containing vermiculite. On emergence of the hypocotyl, 25 seedlings per cultivar were selected for uniformity, and planted, one in each tube. Fifteen plants per cultivar were allocated to one of two treatments, water stress and control. The control plants were watered daily to run-off throughout the experiment. The water stress plants were watered daily to run-off for the first fifteen days from planting, after which they were not watered. All plants were harvested 30 days

after planting. Ten of the 15 tubes per cultivar per treatment were used to measure leaf and root dry weights, and five tubes were used to determine soil water potential.

The plants were grown in a greenhouse where maximum day temperatures varied between 23 and 35°C and minimum night temperatures between 12 and 20°C. The relative humidity varied between 29 and 53%, and PAR was generally between 600 and 950 μmol m^{-2} s^{-1}.

On harvesting, the above-ground portion of each plant was removed. The tube was then laid on its side, the masking tape was cut, and one half of the tube was removed. The soil profile was then sectioned into three equal 20-cm depths and the bulk of the roots removed from each section by washing over a sieve. The roots and above-ground portions of the plants were dried in an oven at 80°C for 48 h, weighed separately, and expressed as dry weight of whole plant, and dry weight of root at different soil depths. Percentage water content on a dry-weight basis of the separate soil sections in the additional tubes was calculated from the weights before and after drying in an oven at 80°C for 48 h. Soil water potentials were then calculated from a curve of percentage soil water content against soil water potential constructed using a pressure plate apparatus.

Water relations

A separate set of plants was used for the physiological measurements of water status and proline and abscisic-acid levels. The same soil mix was used, and seeds were germinated and planted in the same way. Plants were grown in 7-l pots which were watered daily to a constant weight. They were grown in the greenhouse under the same conditions of temperature, humidity and light.

The plants were divided into two treatments: control and water stress. In the water stress treatment, watering was stopped 44 days after planting. When the plants began to wilt after a further eight days, watering was resumed until full recovery after 13 days. The controls were watered normally throughout.

The pots were weighed daily and the dry weight determined at the end of the experiment to calculate percentage water content of the soil. Bulk soil water potentials were then calculated from the curve of percentage soil water content against soil water potential.

Various measurements were done before, during, and on recovery from the water stress on plants in both treatments. The measurements were of transpiration rate, diffusive resistance, relative water content of the leaf, and leaf proline and ABA concentrations.

Transpiration rate and diffusive resistance were measured using a Li-Cor LI-1600 steady-state porometer. Measurements were done at the same time each day, at mid-morning, on the two youngest fully expanded leaves of a plant. These were the sixth and seventh leaves to emerge. Measurements were done on both sides of the leaf. To estimate transpiration rate, the values of both surfaces were added. The data for diffusive resistance are presented here for the abaxial surface only. The mean of these values was calculated for each plant. Each day, five separate plants per cultivar per treatment were measured in this way, and the mean of the means calculated.

Relative water content was measured by the method of Barrs and Weatherley (1962). Three leaf disks per plant were cut from the fifth leaf to emerge, their fresh weight was measured, and they were then floated on distilled water in a covered Petri dish for 2 h, after which the turgid weight was measured. The leaf disks were then dried in an oven and reweighed. Relative water content was calculated by the formula [(fresh weight – dry weight)/(turgid weight – dry weight)] × 100. The mean of these values per plant was calculated, as well as the mean of the means of five plants per treatment per day.

Proline

For determining proline and ABA concentrations, the sixth and seventh leaves to emerge were excised, the midrib was removed, and the two halves of the lamina were weighed, placed in liquid nitrogen and stored separately at –70°C. The two halves were used to determine the proline and ABA concentrations.

Proline concentration was determined by homogenizing the plant material in an extraction medium of 3% aqueous sulfosalicylic acid and measuring the absorbance at 520 nm by the ninhydrin colorimetric procedure (Singh *et al.* 1973). Measurements were done on five plants per cultivar per treatment on six different days.

Abscisic acid

ABA concentration was determined by the method of Hubick and Reid (1980). Samples with a minimum of 1 g of freeze-dried tissue were homogenized with an extraction medium of methanol, ethyl acetate and acetic acid, and then filtered through Whatman No. 1 filter paper. The filtrates were taken to dryness *in vacuo* at 35°C. Further freeze-drying of some of the samples was necessary. 2 ml Dichloromethane was added to the dry sample and 1 ml of the resulting extract was loaded onto a silica Sep-pak cartridge. Contaminants were removed from the sample by a series of organic solvent mixtures before the ABA was eluted. The ABA fractions were bulked, taken to dryness, and the ABA was redissolved in 10 ml 0.5 M phosphate buffer, pH 8.0, and transferred to a new flask. The pH was lowered to 2.5 and the ABA partitioned into ethyl acetate.

The ethyl acetate fraction was taken to dryness and methylated with ethereal diazomethane. The ether was removed under N_2 gas. 0.5 ml ethyl acetate was added to the dry extract and GLC was performed using a Varian 3700 gas chromatograph equipped with a [^{63}Ni] ECD and a 2 m × 3 mm glass column filled with 5% OV-17 on Chromosorb W-HP with N_2 as carrier gas.

Results

Leaf and root growth

In the control treatment the mean whole plant dry weight of the sensitive cultivar was greater (14.6 ± 0.58 g standard error) than that of the resistant cultivar (13.2 ± 0.53 g), though the difference was not significant (Student's *t*-test, $t = 1.94$, $p = 0.068$). In the water stress treatment, the mean whole plant dry weight of the resistant cultivar was significantly greater (7.2 ± 0.33 g) than that of the sensitive cultivar (6.0 ± 0.30 g) ($t = 2.71$, $p = 0.014$). There was a greater reduction in the stress treatment of mean total dry weight of the sensitive cultivar (41% of the control treatment) than the resistant cultivar (55% of the water stress

Table 1 Soil water potential (MPa) of successive 20-cm soil layers in tubes in which the drought-resistant *Zea mays* L. cultivar PNR473 and the drought-sensitive cultivar SR52 were growing after a 15-day period during which tubes were either watered daily to run-off (control treatment) or not watered at all (water stress treatment)[a]

	Treatment and cultivar			
	Control		Water stress	
Soil depth	Resistant	Sensitive	Resistant	Sensitive
0 – 20 cm	–0.056 ± 0.0057	–0.069 ± 0.010	–2.48 ± 0.54	–2.40 ± 0.58
20 – 40 cm	–0.033 ± 0.0031	–0.030 ± 0.0039	–0.72 ± 0.06	–0.84 ± 0.08
40 – 60 cm	–0.025 ± 0.0024	–0.027 ± 0.030	–0.38 ± 0.05	–0.37 ± 0.06

[a] Values are means ± s.e. of five determinations.

treatment). There was no significant difference between the cultivars or the treatments in the root:shoot ratios, which varied between 0.10 and 0.11. In the control treatment, the root dry weight of the resistant cultivar was 1.33 ± 0.052 g and the sensitive cultivar 1.47 ± 0.070 g. In the water stress treatment the root dry weight of the resistant cultivar was 0.79 ± 0.050 g and the sensitive cultivar 0.63 ± 0.045 g.

There was a large difference between the treatments in the soil water potentials in the tubes, but no difference between the cultivars (Table 1). There was a greater proportion of root dry weight in the lowest 20 cm of the soil profile in the water stress treatment than in the control treatment (Figure 1). The proportion of root dry weight in the lowest 20 cm of the soil profile in the stress treatment was greater in the resistant cultivar than in the sensitive cultivar.

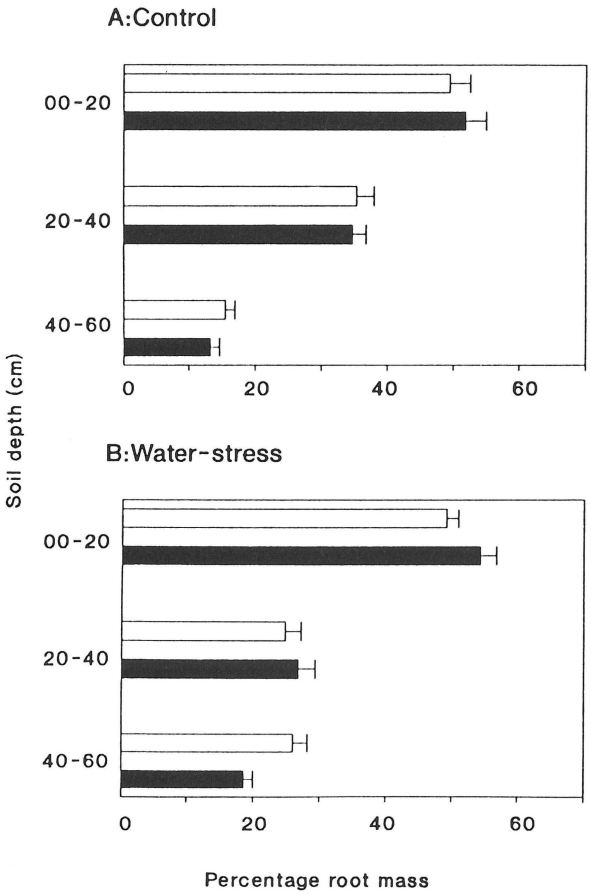

Figure 1 Percentage of total root dry weight of the drought-resistant *Zea mays* L. cultivar PNR473 (open bars) and the drought-sensitive cultivar SR52 (solid bars) in 20-cm soil layers after a 15-day period during which plants were either watered daily (A) or not watered after day 0 (B). Values are means ± s.e. of ten determinations.

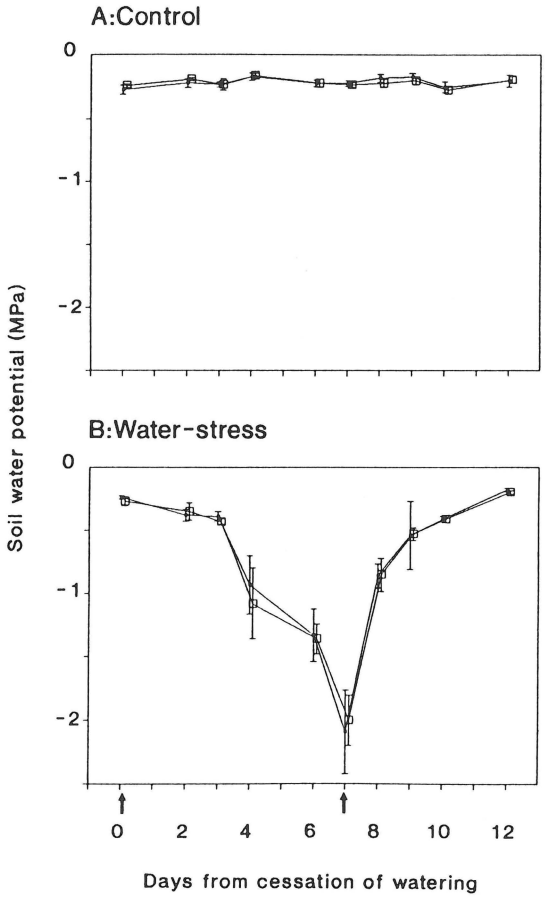

Figure 2 Soil water potential in the pots in which the drought-resistant *Zea mays* L. cultivar PNR473 and the drought-sensitive cultivar SR52 were growing. In the control treatment (A), pots were watered daily to a constant weight. In the water stress treatment (B), watering was stopped when the plants were 40 days old (day 0, arrowed) and resumed when the plants were wilted (day 7, arrowed). Values are means ± s.e. of five determinations. The points (•) represent the resistant cultivar and the open squares (□) the sensitive cultivar.

Water relations

In the pots the soil water potential in the control treatment varied between –0.16 and –0.27 MPa. In the stress treatment, the soil water potential reached –2.09 MPa at peak stress. There was no consistent difference between the cultivars in the soil water potentials (Figure 2).

There was no observable difference between the cultivars in the relative water content in the control treatment. In the stress treatment the relative water content of both cultivars decreased, with that of the sensitive cultivar decreasing at a faster rate and to a lower value at peak stress than that of the resistant cultivar. Recovery to pre-stress levels in both cultivars upon rewatering was rapid (Figure 3).

There was little difference between the cultivars in transpiration rates in the control treatment, although the values for the sensitive cultivar were slightly higher on most days. However, in the stress treatment the transpiration rate of the sensitive cultivar was lowered to a greater extent than that of the resistant cultivar during the onset of stress, until the values were the same at peak stress. Recovery on rewatering was rapid, but the transpiration rates did not reach pre-stress levels. There was no difference between the cultivars in their recovery from water stress (Figure 4).

Diffusive resistances were similar between the two cultivars in the control treatment, with the resistant cultivar having higher values on most days. In the stress treatment the diffusive resistance of the sensitive cultivar increased more rapidly at the onset of stress. Recovery was rapid on rewatering and there was little difference between the cultivars (Figure 5).

Abscisic acid

The concentration of abscisic acid was slightly greater in the sensitive cultivar in the control treatment, but in the stress treatment the concentration of abscisic acid increased to higher levels in the resistant cultivar (Figure 6). There was a significant difference between the cultivars in abscisic acid content at peak stress (Student's t-test, $t = 3.35$, $p = 0.010$).

Proline

There was no difference between the cultivars in the concentration of proline in the control treatment. In the stress treatment there was no change in proline concentration until after day six when there was a rapid increase, and a slow reduction in concentration following rewatering. The proline concentration at the end of the treatment on day 13 was higher than the pre-stress levels. The concentration of

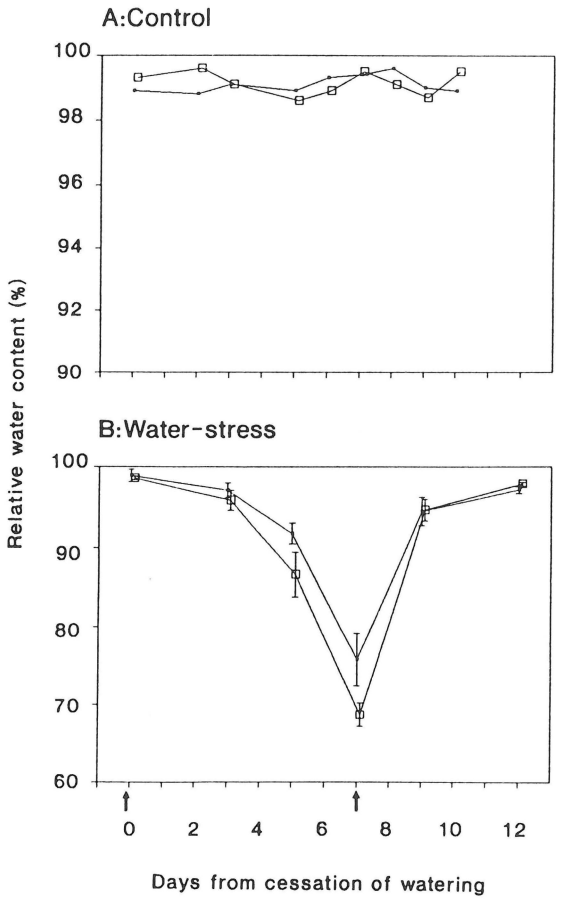

Figure 3 Relative water content of the drought-resistant *Zea mays* L. cultivar PNR473 and the drought-sensitive cultivar SR52. In the control treatment (A), pots were watered daily to a constant weight. In the water stress treatment (B), watering was stopped when the plants were 40 days old (day 0, arrowed) and resumed when the plants were wilted (day 7, arrowed). Values are means ± s.e. of five determinations. The points (•) represent the resistant cultivar and the open squares (□) the sensitive cultivar.

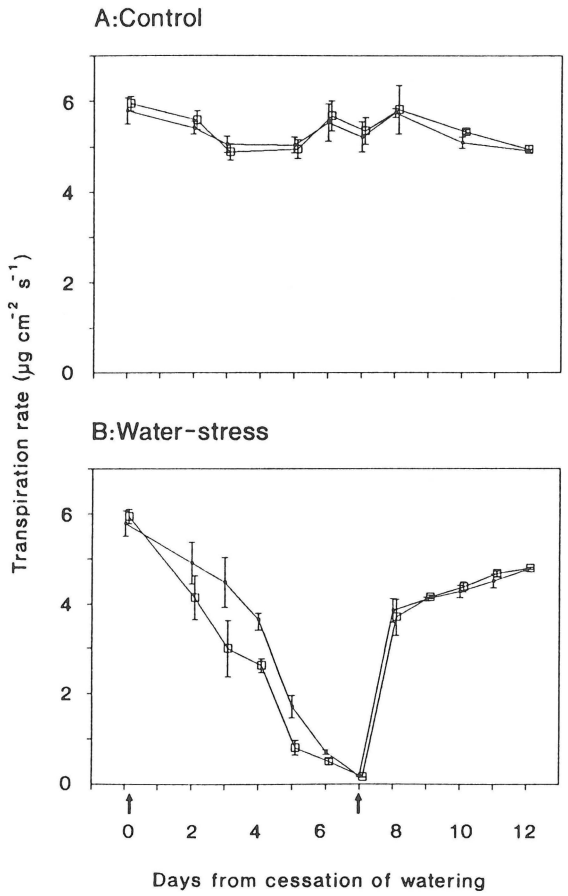

Figure 4 Transpiration rate of the drought-resistant *Zea mays* L. cultivar PNR473 and the drought-sensitive cultivar SR52. In the control treatment (A), pots were watered daily to a constant weight. In the water stress treatment (B), watering was stopped when the plants were 40 days old (day 0, arrowed) and resumed when the plants were wilted (day 7, arrowed). Values are means ± s.e. of five determinations. The points (•) represent the resistant cultivar and the open squares (□) the sensitive cultivar.

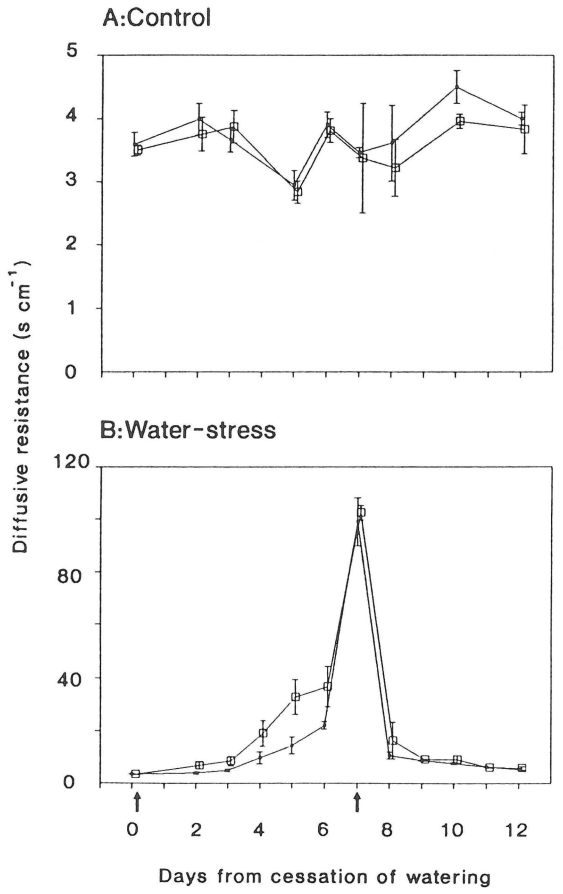

Figure 5 Diffusive resistance of the drought-resistant *Zea mays* L. cultivar PNR473 and the drought-sensitive cultivar SR52. In the control treatment (A), pots were watered daily to a constant weight. In the water stress treatment (B), watering was stopped when the plants were 40 days old (day 0, arrowed) and resumed when the plants were wilted (day 7, arrowed). Values are means ± s.e. of five determinations. The points (•) represent the resistant cultivar and the open squares (□) the sensitive cultivar.

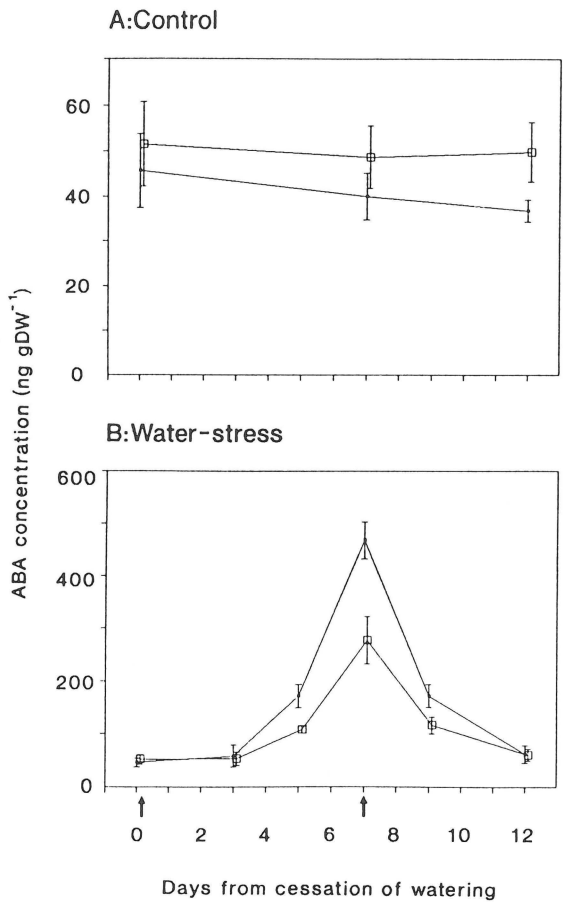

Figure 6 ABA concentration of the drought-resistant *Zea mays* L. cultivar PNR473 and the drought-sensitive cultivar SR52. In the control treatment (A), pots were watered daily to a constant weight. In the water stress treatment (B), watering was stopped when the plants were 40 days old (day 0, arrowed) and resumed when the plants were wilted (day 7, arrowed). Values are means ± s.e. of five determinations. The points (•) represent the resistant cultivar and the open squares (□) the sensitive cultivar.

proline was higher at peak stress in the resistant cultivar (Figure 7). There was a significant difference between the cultivars in proline content at peak stress (Student's *t*-test, $t = 2.74$, $p = 0.025$).

Discussion

The growth measurements confirm that there was a difference between the cultivars in their sensitivity to drought. PNR473 is more resistant than SR52. It was noted that under optimal conditions in the control treatment its growth rate was lower than that of SR52, but in the stress treatment its growth rate was reduced to a lesser extent than that of SR52.

The differences between the cultivars in the various measurements are inherent in the plants and not due to differences in soil water availability since there was no difference between the cultivars in their soil water potentials.

The relative water content of the sensitive cultivar was lower than that of the resistant cultivar in the stress treatment even though the transpiration rate of the sensitive cultivar was lower. This indicated that the sensitive cultivar cannot replace water lost to transpiration under stress conditions as well as the resistant cultivar. This may be accounted for at least partly by the fact that the resistant cultivar has a greater proportion of its roots in the lower soil layer where the soil water potential was higher. Its roots were thus able to extract more water from the soil. An increased effectiveness in supplying water to the leaves by roots deeper in the soil profile when the upper layers have dried, has been noted by other researchers (Sharp & Davies 1985). Similar genotypic differences have been found for wheat where cultivars with greater stress tolerance had more extensive and deeper root systems (Hurd 1974).

The development of soil water deficits can induce deeper rooting (Malik *et al.* 1979). Abscisic acid, the levels of which increase in plants subjected to water stress, can also stimulate the growth of primary root axes (Yamaguchi & Street 1977; Saab *et al.* 1990), thereby increasing the depth to which roots penetrate the soil profile (Watts *et al.* 1981). Increased levels of abscisic acid can also increase hydraulic conductivity of the root (Ludewig *et al.* 1988). It is interesting to note that in this study the resistant cultivar had higher levels of abscisic acid than the sensitive cultivar in the water stress treatment, so it is possible that the same mechanisms of rooting response to abscisic acid are operating.

The result of the changes in rooting characteristics was the maintenance of a higher relative water content and

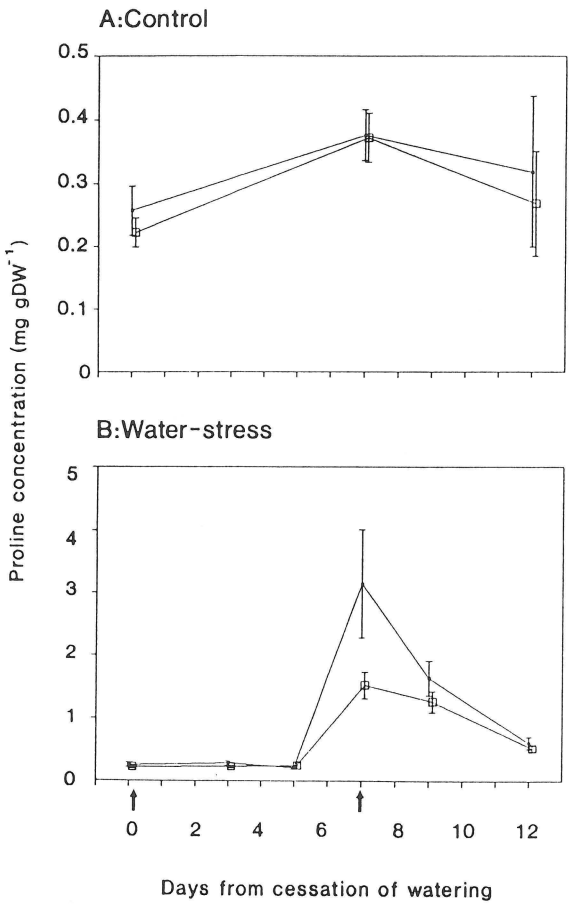

Figure 7 Proline concentration of the drought-resistant *Zea mays* L. cultivar PNR473 and the drought-sensitive cultivar SR52. In the contol treatment (A), pots were watered daily to a constant weight. In the water stress treatment (B), watering was stopped when the plants were 40 days old (day 0, arrowed) and resumed when the plants were wilted (day 7, arrowed). Values are means ± s.e. of five determinations. The points (•) represent the resistant cultivar and the open squares (□) the sensitive cultivar.

transpiration rate and a lower diffusive resistance in the stress-resistant cultivar. These conditions are conducive for continued growth which was reflected in the greater growth of the resistant cultivar compared with the sensitive cultivar in the stress treatment. Although the drought-resistant cultivar had a lower diffusive resistance during the early part of the water stress treatment, both cultivars had complete stomatal closure at the same time. The important difference between the cultivars in this respect was in their ability to withstand the onset of water stress, with the resistant cultivar being able to postpone the onset of tissue desiccation through its deeper rooting response.

At the same soil water potential, the two cultivars are not necessarily suffering the same tissue water stress. Indeed, judging by its relative water content, it appears that the resistant cultivar is experiencing less tissue stress at the same soil water potential than the sensitive cultivar.

The drought-resistant cultivar had a much greater concentration of proline at peak water stress and a rapid recovery to pre-stress levels on rewatering. This pattern has been shown by other researchers (Singh *et al.* 1972; Blum & Ebercon 1976; Hanson *et al.* 1977). The increase in proline levels during water stress appears to enhance a plant's growth on recovery from stress rather than its growth during stress (Blum & Ebercon 1976; Itai & Paleg 1982). Its action during water stress appears to be as a compatible solute to lower the osmotic potential of the cell, and to preferentially hydrate proteins to maintain their tertiary structure at low water potentials (Arakawa & Timasheff 1985).

Acknowledgements

This study was supported by funds from the Foundation for Research Development, the University of Natal Research Fund, the Plant Biotechnology Research Centre and the Water Research Commission.

References

ACKERSON, R.C. 1983. Comparative physiology and water relations of two corn hybrids during water stress. *Crop Sci.* 23: 278 – 283.

ACKERSON, R.C., KRIEG, D.R. & SUNG, F.J.M. 1979. Osmoregulation and leaf conductance of field grown sorghum genotypes. *Crop Sci.* 20: 10 – 14.

ARAKAWA, T. & TIMASHEFF, S.N. 1985. The stabilization of proteins by osmolytes. *Biophys. J.* 47: 411 – 414.

BARRS, H.D. & WEATHERLEY, P.E. 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Aust. J. Biol. Sci.* 15: 413 – 428.

BEADLE, C.L., STEVENSON, K.R., NEUMANN, H.H., THURTELL, G.W. & KING, K.M. 1973. Diffusive resistance, transpiration, and photosynthesis in single leaves of corn and sorghum in relation to leaf water potential. *Can. J. Pl. Sci.* 53: 537 – 544.

BEARDSELL, M.F. & COHEN, D. 1975. Relationships between leaf water status, abscisic acid levels, and stomatal resistance in maize and sorghum. *Pl. Physiol.* 56: 207 – 212.

BLUM, A. & EBERCON, A. 1976. Genotypic responses of sorghum to drought stress III. Free proline accumulation and drought resistance. *Crop Sci.* 16: 428 – 431.

HALL, A.J., LEMCOFF, J.H. & TRAPANI, N. 1981. Water stress before and during flowering in maize and its effects on yield, its components, and their determinations. *Maydica* 26: 19 – 38.

HANSON, A.D., NELSEN, C.E. & EVERSON, E.H. 1977. Evaluation of free proline accumulation as an index of drought resistance using two contrasting barley cultivars. *Crop Sci.* 17: 720 – 726.

HUBICK, K.T. & REID, D.M. 1980. A rapid method for the extraction and analysis of abscisic acid from plant tissue. *Pl. Physiol.* 65: 523 – 525.

HURD, E.A. 1974. Phenotype and drought tolerance in wheat. *Agric. Meteorol.* 14: 39 – 55.

ITAI, C. & PALEG, L.G. 1982. Response of water stressed *Hordeum distichum* L. and *Cucumis sativus* to proline and betaine. *Pl. Sci. Lett.* 25: 329 – 335.

LORENS, G.F., BENNET, J.M. & LOGGALE, L.B. 1987. Differences in drought resistance between two corn hybrids. II. Component analysis of growth rates. *Agron. J.* 79: 808 – 813.

LUDEWIG, M.L., DÖRFFLING, K. & SEIFERT, H. 1988. Abscisic acid and water transport in sunflowers. *Planta* 175: 325 – 333.

MALIK, R.S., DHANKAR, J.S. & TURNER, N.C. 1979. Influence of soil water deficits on root growth in cotton seedlings. *Pl. Soil* 53: 109 – 115.

QUARRIE, S.A. 1980. Genotypic differences in leaf water potential, abscisic acid and proline concentration in spring wheat during drought stress. *Ann. Bot.* 46: 383 – 394.

SAAB, I.N., SHARP, R.E., PRITCHARD, J. & VOETBURG, G.S. 1990. Increased endogenous abscisic acid maintains

primary root growth and inhibits shoot growth of maize seedlings at low water potentials. *Pl. Physiol.* 93: 1329 – 1336.

SHARP, R.E. & DAVIES, W.J. 1985. Root growth and water uptake by maize plants in drying soil. *J. Exp. Bot.* 170: 1441 – 1456.

SINGH, T.N., ASPINALL, D. & PALEG, L.G. 1972. Proline accumulation and varietal adaptability to drought in barley: a potential metabolic measure of drought resistance. *Nature* 236: 188 – 190.

SINGH, T.N., PALEG, L.G. & ASPINALL, R. 1973. Stress metabolism. I. Nitrogen metabolism and growth in the barley plant during water stress. *Aust. J. Biol. Sci.* 26: 45 – 56.

SOBRADO, M.A. 1990. Drought responses of tropical corn 1. Leaf area and yield components in the field. *Maydica* 35: 221 – 226.

THAKUR, P.S. & RAI, V.K. 1981. Growth characteristics and proline content in relation to water status in two *Zea mays* L. cultivars during rehydation. *Biologia Pl.* 23: 98 – 103.

WATTS, S., RODRIGUEZ, J.L., EVANS, S.E. & DAVIES, W.J. 1981. Root and shoot growth of plants treated with abscisic acid. *Ann. Bot.* 47: 595 – 602.

YAMAGUCHI, T. & STREET, H.E. 1977. Stimulation of excised cultured roots of soya bean by abscisic acid. *Ann. Bot.* 41: 1129 – 1133.